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Control of light leaf spot and clubroot in brassica crops using defence elicitors

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Abstract

Plant defence elicitors are compounds that can induce host defence responses against plant pathogens and offer a novel strategy for disease management. Disease control by elicitors can be inconsistent and is often dependent on the crop, the variety and the environment. The use of foliar application of defence elicitors to control light leaf spot (LLS) disease caused by *Pyrenopeziza brassicae* in the brassica crops winter oilseed rape (WOSR) and Brussel sprouts was evaluated in field trials across multiple years. Elicitor responses in WOSR varied between years. Yield benefits were also inconsistent and did not reflect the level of disease control. Results with Brussel sprouts were more consistent although variation between variety, trial site and year were observed. In particular the salicylic acid analog Acibenzolar-S-Methyl, in the commercial product Bion®, demonstrated good disease control across the field trial sites in the early maturing Brussel sprout variety Cobus. Levels of LLS were consistently reduced when Bion® was alternated within a standard fungicide programme, applied as an individual spray or in combination with other defence elicitors. When applied as a root drench or seed soak Bion® also reduced symptom development of the soil-borne brassica disease caused by *Plasmodiophora brassicae*, clubroot, in WOSR. These results indicate that defence elicitors such as Bion® can be used as an additional disease management tool alongside host resistance and standard fungicide programmes to protect brassica crops.

Introduction

The *Brassica* genus contains a range of different crop species that have multiple uses including food for human consumption, animal fodder and vegetable oils. Oilseed rape (OSR; *Brassica napus*) is grown throughout the world as an important source of oil and proteins for animal feed. Furthermore, OSR is important as a break crop in intensive cereal rotations which has resulted in rapeseed oil production increasing more than two-fold in the last ten years in Europe (http://faostat.fao.org/). Vegetable brassica crops are often high value commodities grown for their edible leaves and roots. *Brassica oleracea* is the principle vegetable Brassica crop encompassing cabbages, broccoli, Brussel sprouts and cauliflowers (Rakow, 2004). Frequently OSR and brassica vegetables are grown in close proximity to one another with OSR and vegetable brassica crops grown over approximately 15% of the land in use for arable and horticultural production in the UK in 2014 (Anon., 2015). This can pose issues to the health of these crops as both OSR and vegetable brassica crops are susceptible to many of the same disease threats such as light leaf spot and clubroot.

Light leaf spot (LLS) is an economically important disease of OSR and vegetable brassica crops across Northern Europe (Rawlinson et al., 1978). The disease is caused by the splash-borne fungal pathogen *Pyrenopeziza brassicae* which can lower crop yields by reducing photosynthetic leaf area as well as affecting the quality of vegetable brassica crops (Boys et al., 2007). Varietal resistance to LLS is available in different brassica crops but this is often not sufficient to control the disease (Maddock et al., 1981; Simons and Skidmore, 1988; Boys et al., 2007; Karolewski, 2010) resulting in the widespread use of synthetic fungicides to protect the crops. Fungicides of the methyl benzimidazole carbamate (MBC) and the demethylation inhibitor (DMI) classes have been used alone or in combination to manage LLS. The widespread use of MBC and DMI fungicides to control LLS has resulted in the evolution of *Pyrenopeziza brassicae* isolates that are insensitive to these chemicals (Carter et al., 2013; 2014). The threat posed to LLS control in brassica crops by fungicide insensitive isolates has led to the suggestion that alternative control strategies should be sought for this disease (Carter et al., 2014).

Clubroot is a disease of global importance that affects both broad acre and vegetable brassica crops. Caused by the soil-borne protist, *Plasmodiophora brassicae*, the disease typically results in yield losses of 10-15% although complete crop failures have been associated with severe infection (Dixon, 2009; Hwang et al., 2012). Clubroot symptoms result from

hypertrophic growth of the roots leading to gall formation and deformed roots which can affect the quality value of some brassica vegetables (Dixon, 2009). *Plasmodiophora brassicae* survives as long lived resting spores which can remain viable in the absence of a host for more than fifteen years (Wallenhammar, 1996). The average resting spore half-life of approximately 3.5 years (Wallenhammar, 1996) means control of the disease by rotation is often not a viable option. Different fungicides, biological control agents and soil amendments (Tremblay et al., 2005; Kowata-Dresch and May-De Mio, 2012; Peng et al., 2014; McGrann et al., 2016) have been tested for the control of clubroot but despite some showing activity against *Plasmodiophora brassicae,* their field efficacy is inconsistent and often ineffective (Donald and Porter, 2009). Varietal resistance has provided an effective method of controlling clubroot in different brassica crops but pathogen evolution has resulted in *Plasmodiophora brassicae* populations that can overcome the resistance mechanism (Diederichsen et al., 2009; McGrann et al., 2016; Strelkov et al., 2016).

With fungicides either no longer effective, or declining in efficacy and varietal resistance not completely reliable, alternative control measures for both clubroot and LLS are urgently required. Defence elicitors are compounds that trigger the plants natural defence mechanisms, a process called induced resistance (Walters et al. 2013), and have been shown to provide control against diseases in field grown cereals (Walters et al., 2009; 2011a; 2001b) and brassica crops (Thakur et al., 2014). Oxley and Walters (2012) demonstrated that a combination of the defence elicitors acibenzolar-S-methyl (ASM), cis-jasmone (CJ) and β-aminobutyric acid (BABA) was able to reduce LLS levels in winter oilseed rape (WOSR) more effectively than traditional fungicides. Different elicitors can activate distinct plant defence pathways often regulated by the action of specific plant hormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Walters et al., 2013). Application of the plant hormone SA has been reported to reduce clubroot levels in broccoli (Lovelock et al., 2013) and *Arabidopsis thaliana* (Agarwal et al., 2011) but with negative plant growth effects. These data indicate that compounds that can alter plant hormones and associated defence responses may have potential for use in LLS and clubroot control in brassica crops. The study reported here examined the potential of various defence elicitors to control disease in brassica crops. Field trial experiments assessed the effectiveness of defence elicitors to control LLS in WOSR and Brussel sprouts, whereas glasshouse trials were used to test the effect on clubroot in WOSR.

Methods and materials

Defence elicitors and fungicides

A range of commercially available products were tested for their ability to induce disease resistance in brassica crops. Details of the elicitors and fungicides used in the field trial and glasshouse experiments are provided in Table 1.

Winter oilseed rape (WOSR) field trials

Field trials were used to assess the effects on defence eliciting compounds on reducing LLS disease levels in 2012-13, 2013-14 and 2014-15. Trial design and management was as previously described (Oxley and Walters, 2012) with WOSR varieties sown in a randomised block design at a rate of 60 seeds m^{-2} for a target population size of 50 plants m^{-2} in two adjacent 10 m x 2 m plots with eight rows spaced at 11.5 cm intervals. Local standard practice was followed for all agronomic inputs except for fungicide and elicitor applications. Elicitor and fungicide treatments were applied using a knapsack sprayer in 200 L ha^{-1} of water. There were four replicates of each variety and treatment combination. The 2012-13 trial, at the Bush Estate, Edinburgh, Scotland, examined the effects of Bion® (0.175 g L^{-1} – active ingredient (a.i.) acibenzolar-S-methyl/benzothiadiazole [500 g Kg^{-1}]), and BABA (0.5 g L^{-1} - a.i. DL-b-aminobutyric acid [>95%]) in comparison to the fungicide Folicur (0.5 L ha^{-1} [1] Bayer Crop Science, Cambridge, UK) and untreated control plants of four different WOSR varieties (Table 2). LLS disease resistance ratings from the AHDB (Agricultural and Horticultural Development Board) recommended list (http://cereals.ahdb.org.uk/varieties.aspx) of the four varieties were similar and are noted in parentheses; Excaliber (6), Fashion (6), Flash (5) and Mendel (5). Each treatment was applied twice, once in November 2012 and again in March 2013. In 2013-14 a single WOSR variety, Castille (5), was tested at a trial site in Aberdeen, Scotland. Thirteen treatments were assessed, each applied in November 2013 and again in March 2014 (Table 2). The 2014-15 trial was also located at the Bush Estate, Edinburgh, Scotland and used two WOSR varieties Camelot (5) and PR46W21 (5) and five treatments (Table 2). Treatments were applied in October 2014 and March 2015. Disease levels in all trials were recorded as the percent leaf area infected with LLS four times during the season (Oxley and Walters, 2012). Plot yields at 91% dry matter and the average height of the WOSR plants in each plot were also measured. In 2012-13 and 2014-15 plots were also observed for phytotoxic effects of elicitor or fungicide treatments. Phytotoxicity was visually assessed at 7 and 14 days post

application using a percentage scale where: 0% = no phytotoxicity and 100% = complete foliar necrosis. LLS disease assessments were used to calculate the area under the disease progress curve (AUDPC; Shaner and Finney, 1977) and this value was used for statistical analysis.

Brussel sprout field trials

Field trials were conducted in Scotland in 2013-14 at sites in Tyninghame, East Lothian and Blackness, Falkirk, and in 2015-16 at a site in St Andrews, East Fife. In both years trials were sown in the month of May with three Brussel sprout varieties Cobus, Aurelius and Petrus representing early, mid and late maturing crops, respectively. A series of elicitor treatments were used to assess the effectiveness of five different elicitors applied in four different spray programmes. The five elicitors used were Bion®, Regalia® (a.i. extract of *Reynoutria sachalinensis* [5%]), Softguard® (a.i. chitinosan) [2.6%], SiTKO-SA (a.i. Salicylic acid [4%] and Silica [5%]), and Companion (a.i. Bacillus subtilis GB03 [0.03%]), although Companion was replaced with Alga 600 (soluble seaweed extract powder [40-55% organic matter])in the 2015-16 trial (Table 1; Supplementary Table 1). Elicitor treatments were compared to untreated control plants and to a standard fungicide programme consisting of fungicide applications in mid July, mid August, early September, end of September, mid October and early November. Elicitor treatments were applied as a six spray programme following the same spray timings as for the standard fungicide programme; a three spray programme applied at end July, early September, mid October; a six spray programme where elicitor and fungicide were alternated e.g. elicitor (end July), fungicide (mid August), elicitor (early September), fungicide (end September), elicitor (mid October), fungicide (early November); a three spray programme with elicitors applied in combination applied at end July, early September, mid October. Full details of all 22 treatments are provided in Supplementary Table 1. Local standard practice was followed for all agronomic inputs except for fungicide and elicitor applications. A total of three blocks per treatment with 20 plants in each block were sown. Sprays were applied by knapsack sprayer in 500 L ha^{-1} of water. LLS assessments were made on the lower and top leaves as well as the sprouts on a monthly basis from September until February. During the early assessments (September-November) LLS levels were typically too low for in field scoring. Therefore five samples for each tissue type for all variety and treatment combinations were collected from the field and

incubated in plastic bags for 48 hours at room temperature. Symptoms were scored visually as the percentage of the surface area that was diseased. From December onwards symptoms were visually assessed in the field.

Clubroot glasshouse trials

An isolate of *Plasmodiophora brassicae* was collected from clubroot infested soil from Cupar, Scotland and maintained by serial passage through the susceptible OSR cv. Fashion. Infected galls were collected from susceptible plants, washed free of contaminating soil and stored at -20°C until required. Resting spore suspensions were prepared from frozen galls as previously described (McGrann et al., 2016) and 50 mL of the spore suspension at 2×10^5 spores mL^{-1} added to each 9 x 9 x 8 cm pot filled with compost (John Innes No. 3 compost, John Innes Manufacturers Association, Berkshire, UK) to give a final concentration of 10^4 spores mL^{-1}. WOSR cv. Fashion seeds were sown directly into inoculated soil. Elicitors were applied as a foliar spray using a hand held pump-action sprayer, to run off, or as a 50 mL root drench when WOSR plants were at the 2-3 leaf stage (approximately two weeks after sowing). Elicitors tested included Bion® (0.175 g L^{-1}), Regalia® (5 mL L^{-1}), BABA (1 mM), Companion® (12 mL L^{-1}), SiTKO-SA (10 mL L^{-1}) and Softguard (2 mL L^{-1}). Clubroot was scored on a 0-5 scale based on the severity of galling; 0 = no galling; 1 = small clubs present, most of fibrous root still healthy; 2 = galls visible around tap root and crown; 3 = moderately severe galling with healthy roots still visible; 4 = severe galling with few healthy fibrous roots present; 5 = severe galls with root system now rotten. Clubroot gall fresh weights were also measured in elicitor and control treated plants. The elicitor screen was assessed in three independent experiments. Bion® was also applied as a seed soak to WOSR cv. Fashion by soaking seeds in a solution of Bion® (0.175 g L^{-1}) for 24 hours at 4°C. After 24 hours the seeds were rinsed with distilled water for 10 mins. The effect of soaking seeds with Bion® prior to planting was assessed in two independent experiments and compared control plants grown from seeds soaked in water for 24 hours. Clubroot infection and gall weights were assessed as above 5-8 weeks post inoculation. Plants were observed throughout each experiment for potential growth defects associated with elicitor treatments.

Gene expression assays

Transcripts levels of *Pathogenesis-related 1* (PR1), a known marker of the salicylic acid (SA) defence pathway, were assessed in two distinct experiments following WOSR cv. Fashion treatment with BION®. In the first experiment seeds were sown in pots in clubroot-free compost as described previously and seedlings were treated with a solution of Bion® (0.175 g L^{-1}) as either a foliar spray or as a root drench at the two-three leaf stage approximately two weeks after sowing. Two days later (day 0) each pot was inoculated with a 50 mL (2 x 10^5 spores mL^{-1}) *Plasmodiophora brassicae* resting spore suspension. Leaf samples were collected from three individual plants prior to *Plasmodiophora brassicae* inoculation (day 0) and at 1 and 2 days post inoculation (dpi). For the second experiment clubroot-free compost was inoculated with *Plasmodiophora brassicae* resting spores prior to sowing WOSR seeds, as described previously. Plants were treated with Bion® as in the first experiment and leaf samples collected from three individuals at 1, 2 and 7 days following elicitor treatment. In both experiments samples were also collected from control plants that were not treated with elicitors. In a third experiment WOSR cv. Mendel plants were treated at the two-three leaf stage with Bion® as a foliar spray and one or six days later plants were inoculated with a *Pyrenopeziza brassicae* isolate collected from a WOSR plot in Aberdeen in 2011. *Pyrenopeziza brassicae* was grown on malt extract agar at 16°C for 21-28 days, before spores were collected by flooding the plate with water containing 0.01% Tween 20 and scraping the culture with a spreader. Spores were counted on a haemocytometer and diluted to give a final concentration of 1 x 10^6 spores mL^{-1} and sprayed on to plants to run-off using a hand held pump-action sprayer. Plants were transferred to clear polythene bags post inoculation and incubated at 16°C for 24 hours in the dark followed by 24 hours under a 12 h light:dark photoperiod. The polythene bags were removed 48 hours post inoculation. PR1 expression was assessed in leaf samples 1 and 2 dpi with *Pyrenopeziza brassicae* and compared to untreated control plants.

For gene expression analysis leaf samples were snap frozen in liquid nitrogen after sampling and total RNA extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) followed by Turbo I DNAse (Ambion, Austin, Texas, USA) treatment to remove contaminating genomic DNA. cDNA was synthesised from 1 µg total RNA using the Superscript III first strand cDNA synthesis kit system following the manufacturers instructions (Invitrogen, Carlsbad, CA, USA) and diluted 1 in 50 with sterile distilled water. PR1 transcript levels were measured by quantitative reverse transcription-PCR (qRT-PCR) using the Brilliant II Sybr ® Green qPCR low ROX master mix kit (Agilent Technologies,

Stockport, UK) and gene specific primers PR1_for CACTACACTCAAGTTGTTTGGA and PR1_rev TAGTATGGCTTCTCGTTCACAT. PR1 transcript levels across samples were normalised using primers that amplify the reference gene elongation factor 1α (EF1a) EF1a_for TGAGCACGCTCTTCTTGCTTTCA and EF1a_rev GGTGGTGGCATCCATCTTGTTACA. Relative expression of PR1 in elicitor treated plants was calculated using the $2^{-\Delta\Delta Cq}$ method (Livak and Schmittgen, 2001) with EF1a as the reference gene and control plants not treated with elicitors as the calibrator. qRT-PCR reactions were run using MxPro-Mx3000P 4.10 QPCR System (Agilent Technologies). Each reaction contained 12.5 µL of 2× Brilliant II SYBR Green QPCR Low ROX master mix and 5 µL of cDNA. Final primer concentrations varied on the target gene with PR1_for at 200 nm and PR1_rev at 100 nm whilst EF1a_for and EF1a_rev were both at 300 nm. Reactions were made up to a final volume of 25 µL with sterile distilled water. Thermocycler conditions were as follows: an initial 10 min denaturation step at 95°C followed by 40 cycles of 30s at 95°C; 45 sec at 55°C and 30 sec at 72°C. A dissociation curve was run at the end of each run to confirm that primers were amplifying a single target.

Statistical analyses

All data were analysed using GenStat v15 (Payne et al., 2009). Variation in LLS AUDPC, yield, plant height and phytotoxicity in WOSR field trials was assessed using general linear modelling between years and within years using experimental replicate, treatment and variety as factors where appropriate. Disease symptom data from the Brussel sprout field trials were converted into AUDPC and analysed using a generalized linear model (GLzM) following square root transformation of the AUDPC data to approximate normality. The GLzM assessed variation attributed to block effect, the variety and treatments and any interaction between specific varieties and treatments. Data from the clubroot glasshouse experiments were analysed using general linear modelling using experiment and treatment as factors.

Results

Effect of elicitor treatments on light leaf spot (LLS) in winter oilseed rape (WOSR) field trials

Disease levels were significantly different between the three years (P<0.001) of WOSR field trials with the highest levels of LLS spot recorded in 2014-2015 and the lowest in 2013-14.

In 2012-13 none of the treatments (P = 0.054) nor varieties (P = 0.393) significantly affected LLS AUDPC (Fig. 1a). However, both treatment (P<0.001) and variety (P<0.001) significantly affected yields at 91% dry matter (Fig. 1b). Overall the application of the fungicide Folicur (P = 0.011) and the elicitor Bion® (P<0.001) significantly increased yields compared to control plots. Yields were highest in cv. Flash and Excalibur and lowest in cv. Mendel (Fig. 1b). No significant interaction effect on yield was observed between treatment and variety (P = 0.052). There was no significant effect of treatment on plant height (P = 0.091) but there were differences noted between varieties (P <0.001) with plants of cv. Flash taller than the others (Supplementary Fig. S1a). There were very low levels of phytotoxicity associated with any of the treatments following either of the application dates. None of the treatments had a significant effect on symptoms of phytotoxicity following the Autumn (P = 0.118) or Spring (P = 0.984) applications. There were significantly more phytotoxicity symptoms on the variety Mendel (P = 0.048) following application of the Autumn treatments but this was not linked to any of the treatments and no significant interaction between treatment and variety was observed (P = 0.561).

In 2013-14 there was significant effect of treatment on LLS AUDPC (P <0.001) with all of the treated plots, including those treated with just the adjuvant Warrior, showing lower disease levels compared to the controls (Fig. 1c). There was a significant effect of treatment on yield (P = 0.048), however, despite the reductions in LLS AUDPC associated with all of the treatments tested, only the Bion® (0.175 g L^{-1}) plus Proline (0.35 L ha^{-1}) treatment conferred a significant increase in yield (P = 0.006; Fig. 1d). Treatment also significantly effected plant height (P<0.001) with treatments that included the fungicide Folicur as a component significantly shorter than control and treatments without Folicur (Supplementary Fig. S1b).

In the 2014-15 field trial disease levels appeared lower in treated plots compared to untreated controls. However no significant effect of treatment (P = 0.287) or variety (P = 0.332) on LLS AUDPC was observed (Fig. 1e). There was no effect of treatment on yield in the 2014 trial (P = 0.052) however variety did significantly affect this trait with cv. Camelot typically yielding significantly higher than cv. PR46W21 (P< 0.001; Fig. 1f). Plant height was significantly affected by variety (P< 0.001) with cv. Camelot plants taller than cv. PR46W21 plants (Supplementary Fig. S1c). Treatment also significantly affected plant height (P< 0.001) although there was a significant interaction between variety and treatment (P< 0.001) with all four treatments increasing the height of cv. PR46W21 plants (P< 0.001) but having

no significant effect on cv. Camelot plants. There were no signs of phytotoxicity observed on any of the plants following either the Autumn or Spring application of the treatments (results not shown).

Effector of elicitor treatments on light leaf spot (LLS) in Brussel sprouts field trials

Significant effects ($P < 0.001$) on LLS levels attributable to variety, site and treatment were observed on the lower leaves, top leaves and sprouts in the 2013-14 trial. Disease development was highest on cv. Cobus, followed by cv. Aurelius with little LLS observed on cv. Petrus for all three plant parts scored. LLS development was highest at the Tyninghame site (Supplementary Fig. S2) for the lower leaves and sprouts but disease developed more extensively on the top leaves at Blackness (Supplementary Fig. S3). The different treatments had variable effects of LLS depending on the site, variety and plant part scored. Disease levels scored on either the lower or top leaf or the sprout were not affected by any of the treatments on cv. Aurelius or cv. Petrus. Results on the cv. Cobus were more promising although no single treatment consistently reduced LLS levels on all three scored plant parts at both sites. The standard fungicide programme (treatment (T) 2) provided effective LLS control on lower leaves at both sites but disease control was only observed on the top leaves at Blackness and sprouts at Tyninghame. Of the twenty elicitor-based treatments there was a trend of reduced LLS for treatments that contained Bion® as one of the active ingredients. Alternating Bion® within the fungicide programme (T4) or applied in combination with the elicitors Companion® (T19) or Regalia® (T21) significantly reduced disease in all plant parts at both sites except top leaves at Tyninghame (Fig. 2; Supplementary Fig. S2). Furthermore, the six (T7) and three Bion® spray (T12) programmes were also both effective at lowering LLS on lower leaves at both sites with T7 also effective on top leaves whilst T12 was effective on the sprout at Tyninghame. Although some of the other elicitor treatments had positive effects on LLS control no single ingredient had such a consistent effect as Bion® (Supplementary Fig. S2, S3). Combined Regalia® and SiTKO-SA treatment (T22) increased disease on top leaves at both sites and on lower leaves at Blackness (Supplementary Fig. S2, S3). The three spray SiTKO-SA (T16) programme also increased LLS on sprouts at Blackness (Supplementary Fig. S3).

In the 2015-16 trial, variety and treatment had a significant effect ($P < 0.001$) on LLS AUDPC observed on lower leaf, top leaf and sprouts. Various treatments reduced disease on the different plant parts in cv. Aurelius and cv. Cobus but not on cv. Petrus (Supplementary

Fig. S4). Alternating Bion® within the fungicide programme, six or three spray Bion® treatments or combining Bion® with either Alga® or Regalia® all significantly reduced LLS development in both cv. Aurelius and cv. Cobus (Fig. 2) on all three plant parts assessed (Supplementary Fig. S4). This contrasts with the standard fungicide programme which reduced LLS levels on the sprouts of both varieties but was only effective in significantly lowering disease on the top leaves of cv. Aurelius and the lower leaves of cv. Cobus. In addition six Regalia® sprays (T8) significantly increased disease on the lower leaves. As was seen in 2013-14 some of the other elicitor treatments provided significant reductions in LLS but this control was overall more inconsistent between variety and plant part than seen for the treatments where Bion® was an active ingredient (Supplementary Fig. S4). At all three sites the Bion®-based treatments (T4, T7, T12, T19, T21) were more effective in controlling LLS on the different plant parts in cv. Cobus than the traditional fungicide programme (T2) (Fig. 2).

Effect of elicitor treatments on clubroot in winter oilseed rape (WOSR) glasshouse experiments

Elicitors used against LLS in field trial experiments were assessed in glasshouse assays as potential control agents against clubroot disease of WOSR. Each elicitor was applied as a foliar spray or a root drench. Significant differences in clubroot levels were observed between treatments and experiments and the interactions between the two factors ($P<0.001$). Across the experiments significant reductions in clubroot symptoms were observed on plants treated with Bion® ($P < 0.001$) and SiTKO-SA ($P = 0.003$) drench treatments (Fig. 3a). However, only the Bion® drench treatment significantly reduced the fresh weights of the clubroot galls compared to control plants ($P = 0.003$; Fig. 3b). No obvious growth defects were observed on WOSR plants treated with any elicitor in the experiments (results not shown). Separate experiments were used to assess whether applying Bion® as a seed soak treatment prior to planting could also effect clubroot development in WOSR. Clubroot symptoms were significantly reduced in plants grown from seeds soaked in Bion® ($P<0.001$) compared to control plants (Fig. 3c) and fresh weights of the clubroot galls were significantly lower in the Bion® treated plants ($P = 0.003$; Fig. 3d).

Measurements of Pathogenesis-related protein 1 *(PR1) transcript levels following Bion® treatment*

PR1 transcript levels were increased greater than 10-fold in the leaves of plants two days after treatment with Bion® (day 0) as both a foliar spray and soil drench (Fig. 4a) compared to control plants. This indicates Bion® constitutively activates PR1 expression in WOSR. PR1 expression remained high in Bion® treated plants 1 and 2 dpi with *Plasmodiophora brassicae* resting spores (Fig. 4a). In plants grown for 14 days in *Plasmodiophora brassicae* infested soil prior to Bion® treatment PR1 expression was increased 1, 2 and 7 days following foliar spray or soil drench elicitor treatment compared to untreated controls (Fig. 4b). Plants treated with Bion® either one or six days prior to pathogen inoculation also showed increased PR1 expression 1 and 2 dpi with *Pyrenopeziza brassicae* (Fig. 4c).

Discussion

Sustainable crop productivity is currently under threat due to losses of available disease control options. Chemical control of fungal diseases is at risk from loss of efficacy due to the evolution of fungicide insensitive pathogen isolates (Leadbeater, 2011; Hollomon, 2015). Varietal resistance is limited by a lack of effective, novel sources of resistance against multiple pathogens and the evolution of virulent pathogen isolates (Brown, 2015). The range of crop species in intensive production systems is often limited which exacerbates the disease burden from trash and soil-borne diseases due to close rotations. As such, alternative disease management strategies are required to help maintain adequate levels of control. Defence elicitors that induce plant resistance have been proposed as one control option that could be used to manage disease threats in crops (Walters et al., 2013).

Despite the potential for induced reduced in disease management, a major concern with the use of elicitors is inconsistent levels of disease control and resulting yield benefits (Walters et al., 2013). In the WOSR trials the effects of elicitors on yield did not always correspond to the level of LLS control. In 2012-13 no significant effect of treatment, including the fungicide Folicur, was observed on LLS development yet plots treated with either Folicur or the elicitor Bion® showed an increase in yield (Fig 1a,b). The opposite occurred in 2013-14 where LLS levels were significantly reduced in treated plots compared to the controls but no yield benefit was recorded. Elicitor induced resistance is typically sensitive to varietal variation and environmental conditions (Walters and Fountaine, 2009; Walters et al., 2011a). Such variation in LLS control by various elicitor treatments was observed in both WOSR and Brussel sprouts and was dependent on year, sites and varieties. In particular the late maturing Brussel sprout variety cv. Petrus showed limited response to elicitors compared to the early maturing variety cv. Cobus. This may be related to the lower disease levels observed on Petrus as elicitor-mediated defence typically confers benefits to the crop under high disease pressure (Walters et al., 2009). However, this was not the case in the WOSR trials where the biggest reduction on LLS symptoms was observed in 2013 when disease levels were lowest. It is unclear as to whether or not this discrepancy in elicitor response relates specifically to how these two brassica crops respond to elicitors or is a result of varietal variation in LLS susceptibility (Walters et al., 2011a).

The elicitor that showed the most consistent effects across crops and trials was Bion®. Bion® contains 50% (w/w) acibenzolar-S-methyl (ASM) also called benzothiadiazole (BTH)

which is reported to function as a structural analogue of SA and activate pathways mediated by this plant hormone (Görlach et al., 1996; Ryals et al., 1996). Experiments measuring the expression of the SA marker gene PR1 indicate that treatment with Bion® before or after inoculation with *Plasmodiophora brassicae* or *Pyrenopeziza brassicae* increased levels of PR1 transcript suggesting activation of the SA pathway contributes to induced resistance against these pathogens. SA mediated defence pathways typically function against pathogens with biotrophic development stages (Glazebrook, 2005). *Plasmodiophora brassicae* is an obligate biotroph (Hwang et al., 2012) whereas *Pyrenopeziza brassicae* is classified as a hemibiotroph, with an initial biotrophic infection stage followed by necrotrophic development (Boys et al., 2007). ASM/BTH has been reported to reduce phoma (*Leptosphaeria maculans*) lesions in OSR (Borges et al., 2003; Liu et al., 2006) in glasshouse experiments and Alternaria blight (*Alternaria brassicae*) severity on field grown OSR and Indian mustard (Thakur et al., 2014). Similar to *Pyrenopeziza brassicae*, *Leptosphaeria maculans* and *Alternaria brassicae* have a hemibiotrophic growth habit indicating that ASM/BTH can be effective against fungi with an initial biotrophic growth stage as well as though pathogens that develop specifically biotrophic in nature (Görlach et al., 1996). Additionally ASM/BTH in combination with the defence elicitors cis-jasmone and BABA was shown to effectively lower LLS symptoms in OSR (Oxley and Walters, 2012). Application of SA to brassica plants can reduce clubroot symptoms (Agarwal et al., 2011; Lovelock et al., 2013; Lemarié et al., 2015) whereas recent evidence indicates the *Plasmodiophora brassicae* genome contains a secreted methyltranferase, PbBSMT that can methylate SA. This suggests that *Plasmodiophora brassicae* may be able to modify host SA-mediated defence responses to facilitate pathogen colonisation (Ludwig-Müller et al., 2015). The role of host SA-related pathways in clubroot resistance is further supported by the finding that another SA containing elicitor, SiTKO-SA, also reduced clubroot levels in WOSR. SiTKO-SA contains 4% SA as well as silica which can not only induce defence response but also act as a barrier against pathogen infection (Cai et al., 2009). More effective clubroot control in terms of reduced galling and lower gall weights were observed with Bion®, which contains the high concentration of the SA analog active ingredient compared to SiTKO-SA. High application levels of SA reduce clubroot development in brassica crops but can have detrimental growth effects on the crop (Lovelock et al., 2013). However, no growth defects were observed on the WOSR crops treated with Bion® or SiTKO-SA at the application rates used in these experiments (results not shown).

Clubroot control is inherently difficult due to the soil-borne nature of the disease and the longevity of the resting structures of *Plasmodiophora brassicae*. This is compounded by limited varietal resistance which is under threat from more virulent pathotypes (McGrann et al., 2016; Strelkov et al., 2016). Despite trialling many potential control products, with some showing activity against *Plasmodiophora brassicae* (Kowata-Dresch and May-De Mio, 2012; Peng et al., 2014), the use of chemical control for clubroot is generally inconsistent and often ineffective (Donald and Porter, 2009). Inconsistent clubroot control is also an issue for soil amendments which increase soil pH and soil calcium levels (Tremblay et al., 2005; McGrann et al., 2016). The potential of defence elicitors which affect the host SA pathway opens up new opportunities for the management of clubroot. Although neither Bion® nor SiTKO-SA completed prevented clubroot development, both reduced symptom formation and should be considered as another management tool that can be integrated alongside varietal resistance, rotation and the use of pH raising soil amendments to control this disease.

Other defence elicitors tested in these experiments had more variable effects across the different crops and years particularly on LLS development on Brussel sprouts. Regalia® is a produced from an extract from the plant *Reynoutria sachalinensis* with biofungicidal properties. The extract enhances the plant's defence system through non-systemic induced resistance mediated by increasing phenolics, antioxidants, and strengthening cell walls (Wurms et al., 1999; Fofana et al., 2002). Regalia® and other products formulated from *R. sachalinensis* extracts such as Milsana® are able to effectively control damping-off disease (*Pythium* spp.) in glasshouse produced lettuce (Baysal-Gurel and Miller, 2013), and have shown potential as a component of integrated disease control programmes in organic cucurbit production to reduce powdery mildew (*Podosphaera xanthii*) and downy mildew (*Pseudoperonospora cubensis*) development (Wurms et al., 1999; Fofana et al., 2002; Everts, 2014). Softguard contains chitosan, the deacetylated form N-acetylchitooligosaccharide, a polymer present in fungal cell walls known to trigger defence responses. Products containing chitosan can reduce downy mildew (Sharathchandra et al.2004) in pearl millet when applied as either a foliar spray or seed treatment. The biofungicide Companion® contains *Bascillus subtilis,* which is more commonly associated as a biological control agent due to antimicrobial effects of the bacterium (Fravel, 2005). However, reports have indicated *B. subtilis* can also act as a defence elicitor inducing systemic resistance that can reduce disease in plants (Ongena et al., 2007; Lowe et al., 2012). Despite the reported efficacy of these various defence elicitors to control disease in different crop-pathosystems, results in our

experiments were inconsistent and highly dependent on the trial, variety and part of the plant scored.

In limited cases some of the defence elicitors had a negative impact on disease control. Treatments containing a combination of SiTKO-SA and Regalia® increased LLS on the top leaves of Brussel sprout cv. Cobus plants at both sites in the 2013-14 trial. Combinations of elicitors can result in trade offs in disease resistance against different pathogens. Walters et al. (2011b) demonstrated that a combination of three elicitors, Bion®, BABA and cis-jasmone, was able to effective reduce the levels of powdery mildew (*Blumeria graminis* f. sp. *hordei*) and scald (*Rhynchosporium commune*) but significantly increased levels of Ramularia leaf spot (*Ramularia collo-cygni*) in field trials of two barley varieties. Why the combined treatment of SiTKO-SA and Regalia® or these two elicitors as individual components increased LLS in the some of the trials is unclear. Antagonism between defence pathways is well known in plants, particularly the pathways regulated by plant hormones SA and JA/ET involved in defence against biotrophic and necrotrophic pathogens (Glazebrook, 2005). As it is likely that different elicitors target specific induced resistance pathways then altered cross-talk between signalling pathways within the plant could lead to antagonistic interactions such that combining certain elicitors may result in potential trade offs in disease resistance and increased pathogen development (Walters et al., 2011b).

Declines in fungicide efficacy against LLS in brassica crops (Carter et al., 2013; 2014) together with the threat of reduced fungicide availability in the long term (Leadbeater, 2011) has resulted in a need to protect those fungicides that still effectively control disease. Integrated disease management strategies focussed on using alternative control measures to manage crop diseases in order to lower fungicide inputs can help prolong the effective shelf life of fungicides by reducing the risk of fungicide insensitivity and limit evolution in pathogen populations (Hollomon, 2015). Alternating Bion® within the fungicide programme in the Brussel sprout field trials (treatment 4) or using a reduced fungicide rate in combination with Bion® in the 2013-14 WOSR trial effectively reduced LLS levels. Furthermore, treatments which alternated other elicitors, such as Regalia®, Softguard and Companion with fungicides, were able to lower disease levels, although not as consistently as Bion®. Together these results suggest that elicitors may have a useful role as plant protection products going forward to help delay development of fungicide insensitivity. Furthermore, using elicitors in conjunction with clubroot resistant brassica crops may also help prevent erosion of the limited resistance sources to this disease which are under threat

from resistance-breaking isolates of *Plasmodiophora brassicae* (McGrann et al., 2016; Strelkov et al., 2016). The results presented here are promising but to realise the full potential of these compounds and to implement defence elicitors within disease management programmes a more comprehensive understanding of how specific defence elicitors affect plant defence pathways that operate against different pathogens and how induced resistance is influenced by environmental conditions and host genetics is required.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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655 **Tables**

656 Table 1 Details of defence elicitors and fungicides used in this study

	Active ingredient	**Company**
Defence elicitors		
Bion®	Acibenzolar-S-methyl/benzothiadiazole (500 g Kg^{-1})	Syngenta, Jealott's Hill, UK
Regalia® Biofungicide	Extract of *Reynoutria sachalinensis* (5%)	Syngenta, Jealott's Hill, UK
BABA	DL-b-aminobutyric acid (>95%)	Sigma, Dorset, UK
Companion ®	Bacillus subtilis GB03 (0.03%)	Growth products, USA
SiTKO-SA	Salicylic acid (4%), Silica (5%)	Growth products, USA
Softguard	Chitinosan (2.6%)	Travena, UK
Alga 600	Soluble Seaweed extract powder (40-55% organic matter)	Travena, UK
Fungicides		
Folicur ®	250 g L^{-1} (25.9% w/w) tebuconazole	Bayer CropScience, Cambridge, UK
Proline 275 ®	275 g L^{-1} (27.5% w/w) prothioconazole	Bayer CropScience, Cambridge, UK
Signum ®	26.7% w/w boscalid and 6.7% w/w pyroclostrobin	BASF,
Rudis ®	480 g/L (40 % w/w) prothioconazole	Bayer CropScience, Cambridge, UK
Nativo 75WG ®	250 g Kg^{-1} (25.0 % w/w)trifloxystrobin and 500 g Kg^{-1} (50.0% w/w) tebuconazole	Bayer CropScience, Cambridge, UK
Adjuvant		
Warrior	192 g L^{-1} primary alcohol ethoxylate	Intracrop ©, Gloucestershire, UK

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659 Table 2 Treatments used in WOSR field trials 2012-13, 2013-14 and 2014-15

	Treatments		
	2012-2013	**2013-14**	**2014-15**
1	Untreated (control)	Untreated (control)	Untreated (control)
2	Folicur (0.5 L ha^{-1})	Folicur (0.7 L ha^{-1})	Folicur (0.7 L ha^{-1})
3	Bion® (0.175 g L^{-1})	Bion® (0.175 g L^{-1})	Bion® (0.175 g L^{-1}) + Warrior (25 mL 100 L^{-1})
4	BABA (0.5 g L^{-1})	BABA (0.5 g L^{-1})	BABA (0.5 g L^{-1}) + Warrior (25 mL 100 L^{-1})
5		Folicur (0.5 L ha^{-1})	Regalia (2.5 L ha^{-1}) + Warrior (25 mL 100 L^{-1})
6		Bion® (0.175 g L^{-1}) + Folicur (0.5 L ha^{-1})	
7		BABA (0.5 g L^{-1}) + Folicur (0.5 L ha^{-1})	
8		Folicur (0.5 L ha^{-1}) + Warrior (25 mL 100 L^{-1})	
9		Bion® (0.175 g L^{-1}) + Proline (0.35 L ha^{-1})	
10		Proline (0.35 L ha^{-1})	
11		Proline (0.5 L ha^{-1})	
12		Bion® (0.175 g L^{-1}) + Folicur (0.7 L ha^{-1})	
13		Warrior (25 mL 100 L^{-1})	

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Figure legends

Fig. 1 Field performance of elicitor treatments on winter oilseed rape (WOSR) crops. Effects of elicitors on light leaf spot development measured as the area under the disease progress curve (AUDPC) in 2012-13 (a), 2013-14 (c), 2014-15 (e) and WOSR yield at 91% dry matter in 2012-13 (b), 2013-14 (d), 2014-15 (f). Bars indicate standard error. *** = P <0.001; ** = P <0.01; * = P <0.05.

Fig. 2 Field performance of elicitor treatments containing Bion® as a component on the Brussel sprout cv. Cobus. Effects of elicitor treatments on light leaf spot development measured as the area under the disease progress curve (AUDPC) on the lower leaves (a), top leaves (b) and sprouts (c). Trials were run at sites in Scotland at Blackness and Tyninghame in 2013-14 and in St Andrews in 2015-16. Treatments were T1 = Untreated controls; T2 = standard fungicide programme; T4 = Bion® alternated within the standard fungicide programme; T7 = six Bion® sprays; T12 = three Bion® sprays; T19 = three Bion + Companion/Alga 600 (2013-14/2015-16) sprays; T21 = three Bion® + Regalia® sprays. Bars indicate standard error. *** = P <0.001; ** = P <0.01; * = P <0.05.

Fig. 3 Effect of elicitor compounds on clubroot development in glasshouse conditions. Elicitors were applied as a foliar spray or root drench and the effect on clubroot symptom development (a) and gall fresh weight (b) was assessed after 5-8 weeks growth infested soil. Effect of Bion® as a seed soak on clubroot development (c) and gall fresh weight (d). Bars indicate standard error. *** = P <0.001; ** = P <0.01; * = P <0.05.

Fig. 4 Effect of Bion® on *Pathogenesis-related 1* (PR1) transcript levels in winter oilseed rape (WOSR). (a) PR1 levels in Bion treated WOSR plants at 0, 1 and 2 days post inoculation (dpi) with *Plasmodiophora brassiace*. (b) PR1 levels in WOSR grown in clubroot infested soil and then treated with Bion®. Transcript levels measured 1, 2, and 7 days post Bion® treatment. (c) PR1 levels in WOSR plants treated with Bion® either one or six days prior to inoculation with *Pyrenopeziza brassicae*. Transcript levels measured 1 and 2 dpi with *Pyrenopeziza brassicae*. PR1 transcript levels are normalised to the reference genes elongation factor 1-α and data is presented as fold change relative to the elicitor control (water)-treated samples. Bars indicate standard error.

Electronic Supplementary Material

Supplementary Table 1 Treatments used in Brussel sprout field trials 2013-14 and 2015-16

Supplementary Fig. 1. Effects of elicitors on winter oilseed rape height in field trials in 2012-13 (a), 2013-14 (b), 2014-15 (c). Bars indicate standard error.

Supplementary Fig. 2. Field performance of elicitor treatments on light leaf spot development on Brussel sprout varieties at Tyninghame site in 2013-14.

Supplementary Fig. 3. Field performance of elicitor treatments on light leaf spot development on Brussel sprout varieties at Blackness site in 2013-14.

Supplementary Fig. 4. Field performance of elicitor treatments on light leaf spot development on Brussel sprout varieties at Blackness site in 2015-16.

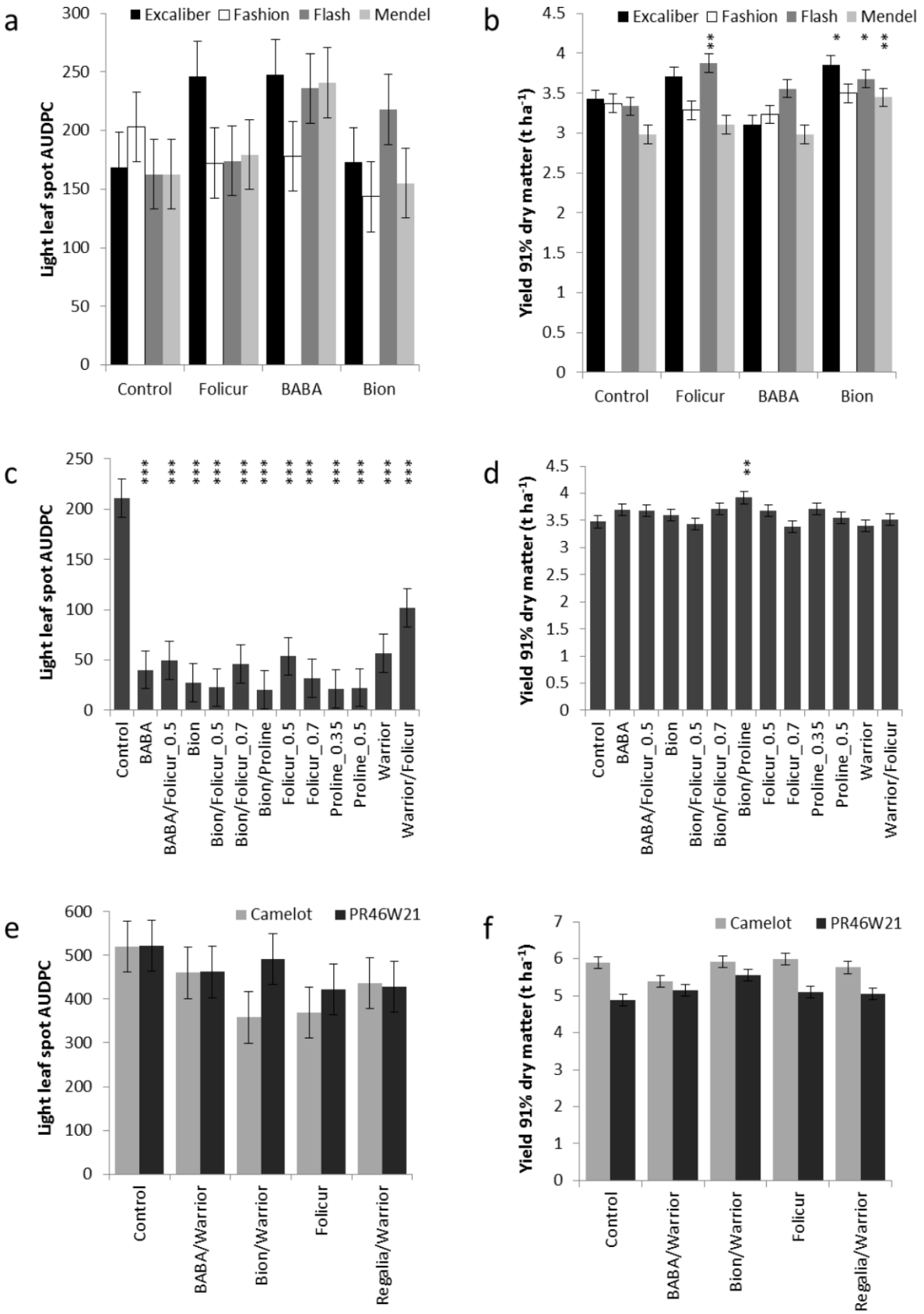
a
Excaliber
Fashion
Flash
Mendel
Light leaf spot AUDPC
Control
Folicur
BABA
Bion
b
Yield 91% dry matter (t ha-1)
c
Control
BABA
BABA/Folicur_0.5
Bion
Bion/Folicur_0.5
Bion/Folicur_0.7
Bion/Proline
Folicur_0.5
Folicur_0.7
Proline_0.35
Proline_0.5
Warrior
Warrior/Folicur
d
e
Camelot
PR46W21
BABA/Warrior
Bion/Warrior
Regalia/Warrior
f

Fig. 1

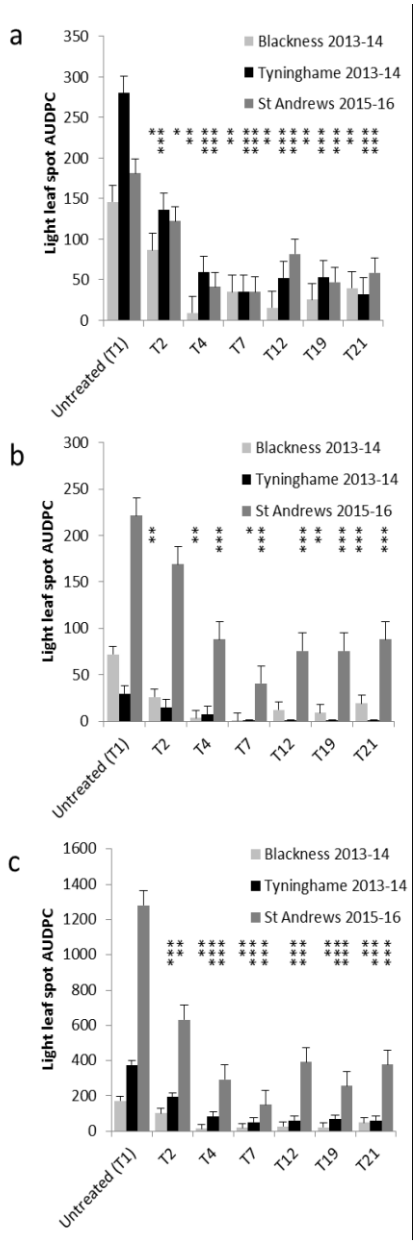
a
Light leaf spot AUDPC
Blackness 2013-14
Tyninghame 2013-14
St Andrews 2015-16
Untreated (T1)
T2
T4
T7
T12
T19
T21
b
Light leaf spot AUDPC
Blackness 2013-14
Tyninghame 2013-14
St Andrews 2015-16
Untreated (T1)
T2
T4
T7
T12
T19
T21
c
Light leaf spot AUDPC
Blackness 2013-14
Tyninghame 2013-14
St Andrews 2015-16
Untreated (T1)
T2
T4
T7
T12
T19
T21

Fig. 2

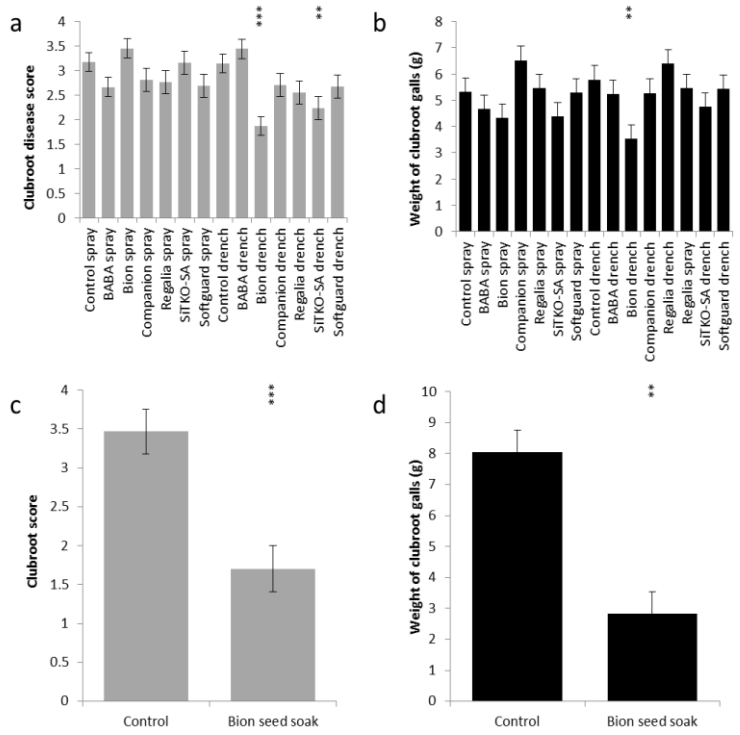
a
Clubroot disease score
Control spray
BABA spray
Bion spray
Companion spray
Regalia spray
SITKO-SA spray
Softguard spray
Control drench
BABA drench
Bion drench
Companion drench
Regalia drench
SITKO-SA drench
Softguard drench
b
Weight of clubroot galls (g)
c
Clubroot score
Control
Bion seed soak
d
Weight of clubroot galls (g)

Fig. 3

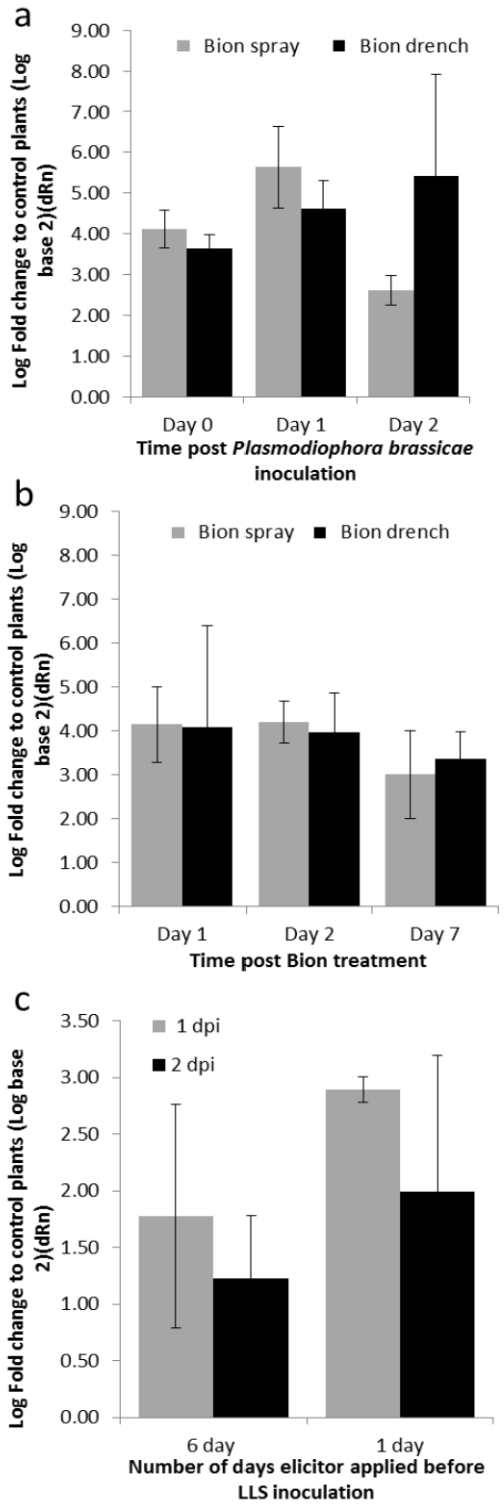
a
Log Fold change to control plants (Log base 2)(dRn)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Bion spray
Bion drench
Day 0
Day 1
Day 2
Time post Plasmodiophora brassicae inoculation
b
Log Fold change to control plants (Log base 2)(dRn)
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Bion spray
Bion drench
Day 1
Day 2
Day 7
Time post Bion treatment
c
Log Fold change to control plants (Log base 2)(dRn)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1 dpi
2 dpi
6 day
1 day
Number of days elicitor applied before LLS inoculation

Fig. 4

Table S1 Treatments used in Brussel sprout field trials 2013-14 and 2015-16

Treatment	Mid July	End July	Mid August	Early September	End September	Mid October	Early November
1	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated
2	Signum 1 Kg ha^{-1}		Rudis 0.4 L ha^{-1}	Nativo 0.4 Kg ha^{-1}	Signum 1 Kg ha^{-1}	Rudis 0.4 L ha^{-1}	Nativo 0.4 Kg ha^{-1}
3		Regalia 2.5 L ha^{-1}	Rudis 0.4 L ha^{-1}	Regalia 2.5 L ha^{-1}	Signum 1 Kg ha^{-1}	Regalia 2.5 L ha^{-1}	Nativo 0.4 Kg ha^{-1}
4		Bion 0.175 g L^{-1}	Rudis 0.4 L ha^{-1}	Bion 0.175 g L^{-1}	Signum 1 Kg ha^{-1}	Bion 0.175 g L^{-1}	Nativo 0.4 Kg ha^{-1}
5		Softguard 10 mL $5L^{-1}$	Rudis 0.4 L ha^{-1}	Softguard 10 mL $5L^{-1}$	Signum 1 Kg ha^{-1}	Softguard 10 mL $5L^{-1}$	Nativo 0.4 Kg ha^{-1}
6		SiTKO-SA 5 L ha^{-1}	Rudis 0.4 L ha^{-1}	SiTKO-SA 5 L ha^{-1}	Signum 1 Kg ha^{-1}	SiTKO-SA 5 L ha^{-1}1	Nativo 0.4 Kg ha^{-1}
7		Bion 0.175 g L^{-1}	Bion 0.175 g L^{-1}	Bion 0.175 g L^{-1}	Bion 0.175 g L^{-1}	Bion 0.175 g L^{-1}	Bion 0.175 g L^{-1}
8		Regalia 2.5 L ha^{-1}	Regalia 2.5 L ha^{-1}	Regalia 2.5 L ha^{-1}	Regalia 2.5 L ha^{-1}	Regalia 2.5 L ha^{-1}	Regalia 2.5 L ha^{-1}
9		Softguard 10 mL $5L^{-1}$	Softguard 10 mL $5L^{-1}$	Softguard 10 mL $5L^{-1}$	Softguard 10 mL $5L^{-1}$	Softguard 10 mL $5L^{-1}$	Softguard 10 mL $5L^{-1}$
10		Alga 600 3 g $5L^{-1}$ [a]	Alga 600 3 g $5L^{-1}$	Alga 600 3 g $5L^{-1}$	Alga 600 3 g $5L^{-1}$	Alga 600 3 g $5L^{-1}$	Alga 600 3 g $5L^{-1}$
11		SiTKO-SA 5 L ha^{-1}	SiTKO-SA 5 L ha^{-1}	SiTKO-SA 5 L ha^{-1}	SiTKO-SA 5 L ha^{-1}	SiTKO-SA 5 L ha^{-1}	SiTKO-SA 5 L ha^{-1}
12		Bion 0.175 g L^{-1}		Bion 0.175 g L^{-1}		Bion 0.175 g L^{-1}	
13		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}	
14		Softguard 10 mL $5L^{-1}$		Softguard 10 mL $5L^{-1}$		Softguard 10 mL $5L^{-1}$	
15		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$	
16		SiTKO-SA 5 L ha^{-1}		SiTKO-SA 5 L ha^{-1}		SiTKO-SA 5 L ha^{-1}	
17		Softguard 10 mL $5L^{-1}$		Softguard 10 mL $5L^{-1}$		Softguard 10 mL $5L^{-1}$	
		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$	
18		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}	
		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$	
19		Bion 0.175 g L^{-1}		Bion 0.175 g L^{-1}		Bion 0.175 g L^{-1}	
		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$	
20		SiTKO-SA 5 L ha^{-1}		SiTKO-SA 5 L ha^{-1}		SiTKO-SA 5 L ha^{-1}	
		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$		Alga 600 3 g $5L^{-1}$	
21		Bion 0.175 g L^{-1}		Bion 0.175 g L^{-1}		Bion 0.175 g L^{-1}	
		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}	
22		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}		Regalia 2.5 L ha^{-1}	
		SiTKO-SA 5 L ha^{-1}		SiTKO-SA 5 L ha^{-1}		SiTKO-SA 5 L ha^{-1}	

[a] In 2013-14 Treatment 10, 15, 17, 18, 19, 20 Companion 6L ha^{-1} was used in place of Alga 600 3 g $5L^{-1}$.

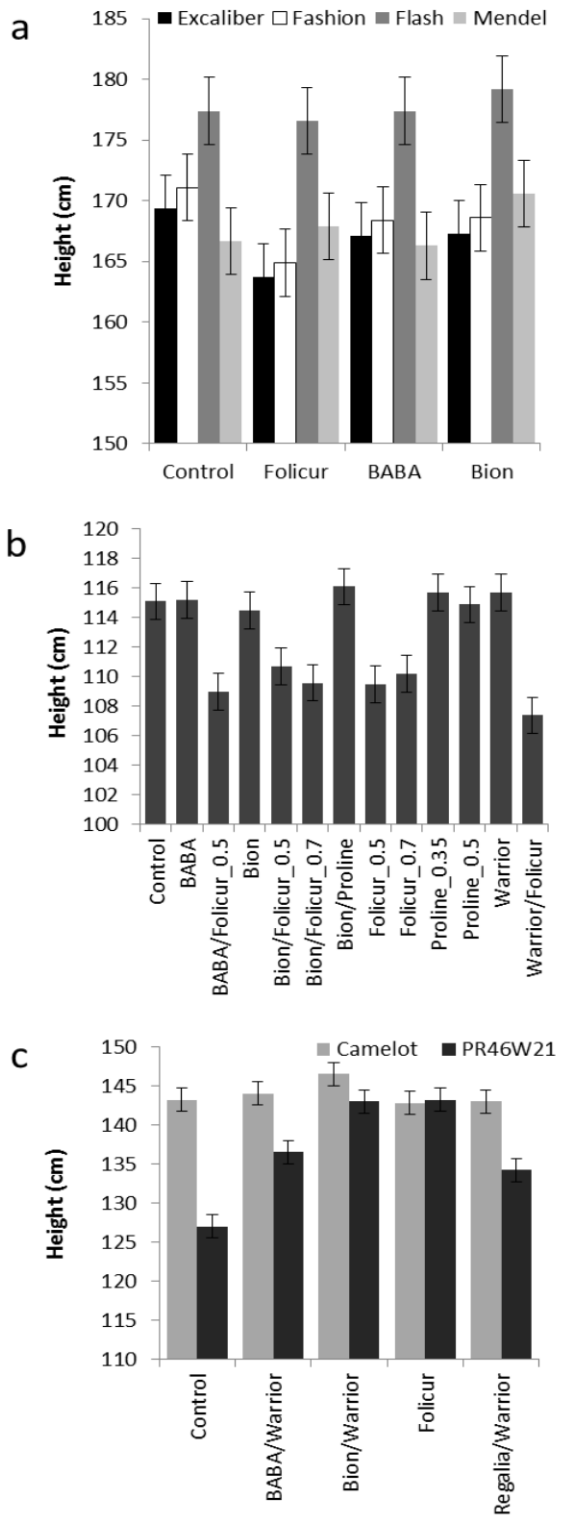

Figure S1. Effects of elicitors on winter oilseed rape height in field trials in 2012-13 (a), 2013-14 (b), 2014-15 (c). Bars indicate standard error.

Supplementary Figure 1

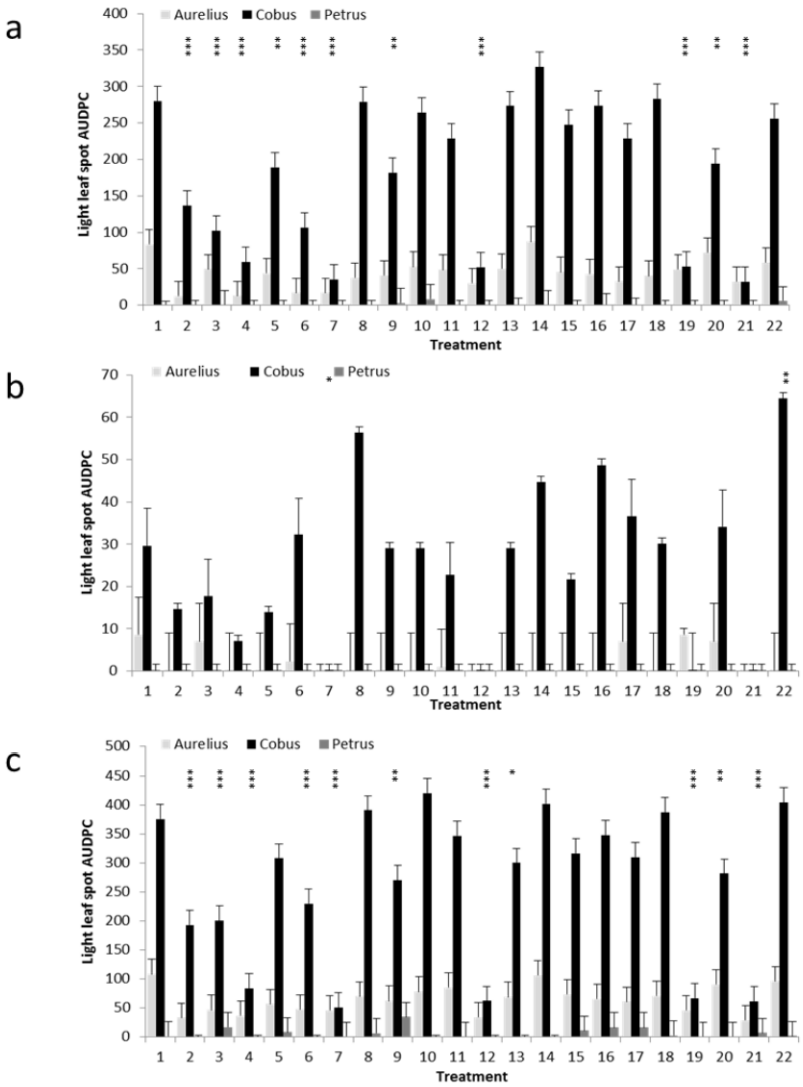

Figure S2. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Tyninghame site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated: Treatment 2 = Mid July Signum 1 Kg ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Nativo 0.4 Kg ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Rudis 0.4 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 3 = End July Regalia 2.5 L ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Regalia 2.5 L ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Regalia 2.5 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 4 = End July Bion 0.175 g L^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Bion 0.175 g L^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Bion 0.175 g L^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 5 = End July Softguard 10 mL $5L^{-1}$; Mid August Rudis 0.4 L ha^{-1}; Early September Softguard 10 mL $5L^{-1}$; End September Signum 1 Kg ha^{-1}; Mid October Softguard 10 mL $5L^{-1}$; Early November Nativo 0.4 Kg ha^{-1}: Treatment 6 = End July SiTKO-SA 5 L ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September SiTKO-SA 5 L ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October SiTKO-SA 5 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 7 = End July, Mid August, Early September, End September, Mid October, Early November Bion 0.175 g L^{-1}: Treatment 8 = End July, Mid August, Early September, End September, Mid October, Early November Regalia 2.5 L ha^{-1}: Treatment 9 = End July, Mid August, Early September, End September, Mid October, Early November Softguard 10 mL $5L^{-1}$: Treatment 10 = End July, Mid August, Early September, End September, Mid October, Early November Companion 6L ha^{-1} : Treatment 11 = End July, Mid August, Early September, End September, Mid October, Early November SiTKO-SA 5 L ha^{-1}: Treatment 12 = End July, Early September, Mid October, Bion 0.175 g L^{-1}: Treatment 13 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1}: Treatment 14 = End July, Early September, Mid October, Softguard 10 mL $5L^{-1}$: Treatment 15 = End July, Early September, Mid October, Companion 6L ha^{-1} : Treatment 16 = End July, Early September, Mid October, SiTKO-SA 5 L ha^{-1}: Treatment 17 = End July, Early September, Mid October, Softguard 10 mL $5L^{-1}$ & Companion 6L ha^{-1} : Treatment 18 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1} & Companion 6L ha^{-1} : Treatment 19 = End July, Early September, Mid October, Bion 0.175 g L^{-1} & Companion 6L ha^{-1} : Treatment 20 = End July, Early September, Mid October, SiTKO-SA 5 L ha^{-1} & Companion 6L ha^{-1} : Treatment 21 = End July, Early September, Mid October, Bion 0.175 g L^{-1} & Regalia 2.5 L ha^{-1}: Treatment 22 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1} & SiTKO-SA 5 L ha^{-1}. *** = P <0.001; ** = P <0.01; * = P <0.05

Supplementary Figure 2

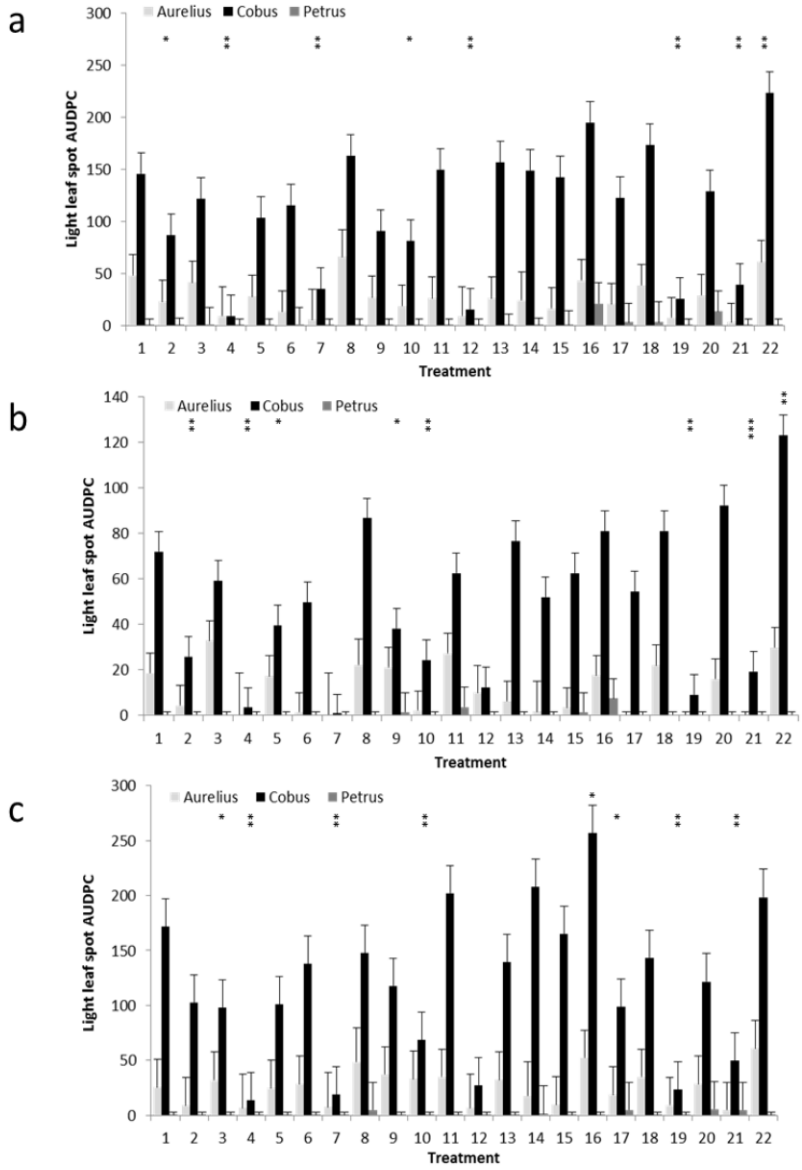

Figure S3. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at Blackness site in 2013-14. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated: Treatment 2 = Mid July Signum 1 Kg ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Nativo 0.4 Kg ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Rudis 0.4 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 3 = End July Regalia 2.5 L ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Regalia 2.5 L ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Regalia 2.5 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 4 = End July Bion 0.175 g L^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Bion 0.175 g L^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Bion 0.175 g L^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 5 = End July Softguard 10 mL $5L^{-1}$; Mid August Rudis 0.4 L ha^{-1}; Early September Softguard 10 mL $5L^{-1}$; End September Signum 1 Kg ha^{-1}; Mid October Softguard 10 mL $5L^{-1}$; Early November Nativo 0.4 Kg ha^{-1}: Treatment 6 = End July SiTKO-SA 5 L ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September SiTKO-SA 5 L ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October SiTKO-SA 5 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 7 = End July, Mid August, Early September, End September, Mid October, Early November Bion 0.175 g L^{-1}: Treatment 8 = End July, Mid August, Early September, End September, Mid October, Early November Regalia 2.5 L ha^{-1}: Treatment 9 = End July, Mid August, Early September, End September, Mid October, Early November Softguard 10 mL $5L^{-1}$: Treatment 10 = End July, Mid August, Early September, End September, Mid October, Early November Companion 6L ha^{-1} : Treatment 11 = End July, Mid August, Early September, End September, Mid October, Early November SiTKO-SA 5 L ha^{-1}: Treatment 12 = End July, Early September, Mid October, Bion 0.175 g L^{-1}: Treatment 13 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1}: Treatment 14 = End July, Early September, Mid October, Softguard 10 mL $5L^{-1}$: Treatment 15 = End July, Early September, Mid October, Companion 6L ha^{-1} : Treatment 16 = End July, Early September, Mid October, SiTKO-SA 5 L ha^{-1}: Treatment 17 = End July, Early September, Mid October, Softguard 10 mL $5L^{-1}$ & Companion 6L ha^{-1} : Treatment 18 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1} & Companion 6L ha^{-1} : Treatment 19 = End July, Early September, Mid October, Bion 0.175 g L^{-1} & Companion 6L ha^{-1} : Treatment 20 = End July, Early September, Mid October, SiTKO-SA 5 L ha^{-1} & Companion 6L ha^{-1} : Treatment 21 = End July, Early September, Mid October, Bion 0.175 g L^{-1} & Regalia 2.5 L ha^{-1}: Treatment 22 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1} & SiTKO-SA 5 L ha^{-1}. *** = P <0.001; ** = P <0.01; * = P <0.05

Supplementary Figure 3

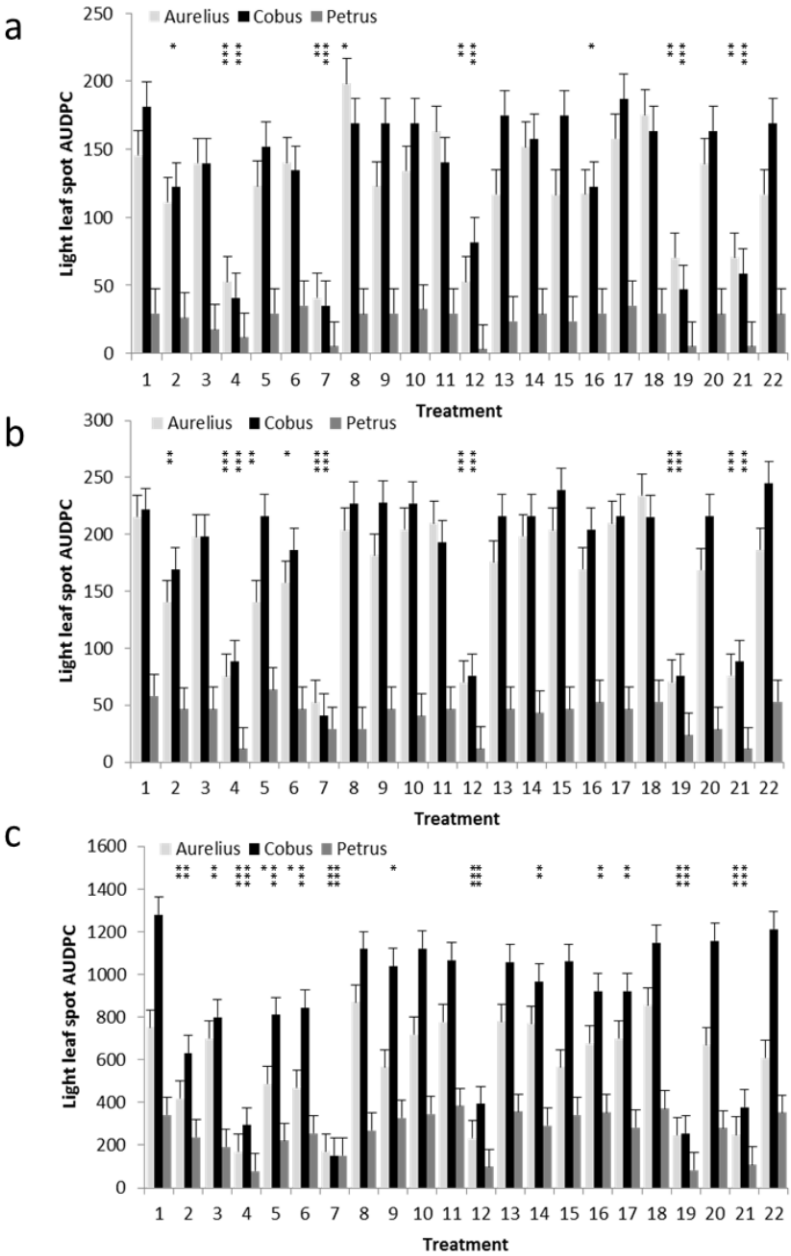

Figure S4. Field performance of elicitor treatments on light leaf spot (LLS) development on Brussel sprout varieties at St Andrews site in 2015-16. LLS development measured as the area under the disease progress curve (AUDPC) lower leaves (a), top leaves (b) and sprouts (c). Treatments were as follows: Treatment 1 = untreated: Treatment 2 = Mid July Signum 1 Kg ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Nativo 0.4 Kg ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Rudis 0.4 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 3 = End July Regalia 2.5 L ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Regalia 2.5 L ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Regalia 2.5 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 4 = End July Bion 0.175 g L^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September Bion 0.175 g L^{-1}; End September Signum 1 Kg ha^{-1}; Mid October Bion 0.175 g L^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 5 = End July Softguard 10 mL $5L^{-1}$; Mid August Rudis 0.4 L ha^{-1}; Early September Softguard 10 mL $5L^{-1}$; End September Signum 1 Kg ha^{-1}; Mid October Softguard 10 mL $5L^{-1}$; Early November Nativo 0.4 Kg ha^{-1}: Treatment 6 = End July SiTKO-SA 5 L ha^{-1}; Mid August Rudis 0.4 L ha^{-1}; Early September SiTKO-SA 5 L ha^{-1}; End September Signum 1 Kg ha^{-1}; Mid October SiTKO-SA 5 L ha^{-1}; Early November Nativo 0.4 Kg ha^{-1}: Treatment 7 = End July, Mid August, Early September, End September, Mid October, Early November Bion 0.175 g L^{-1}: Treatment 8 = End July, Mid August, Early September, End September, Mid October, Early November Regalia 2.5 L ha^{-1}: Treatment 9 = End July, Mid August, Early September, End September, Mid October, Early November Softguard 10 mL $5L^{-1}$: Treatment 10 = End July, Mid August, Early September, End September, Mid October, Early November Alga 600 3 g $5L^{-1}$: Treatment 11 = End July, Mid August, Early September, End September, Mid October, Early November SiTKO-SA 5 L ha^{-1}: Treatment 12 = End July, Early September, Mid October, Bion 0.175 g L^{-1}: Treatment 13 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1}: Treatment 14 = End July, Early September, Mid October, Softguard 10 mL $5L^{-1}$: Treatment 15 = End July, Early September, Mid October, Alga 600 3 g $5L^{-1}$: Treatment 16 = End July, Early September, Mid October, SiTKO-SA 5 L ha^{-1}: Treatment 17 = End July, Early September, Mid October, Softguard 10 mL $5L^{-1}$ & Alga 600 3 g $5L^{-1}$: Treatment 18 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1} & Alga 600 3 g $5L^{-1}$: Treatment 19 = End July, Early September, Mid October, Bion 0.175 g L^{-1} & Alga 600 3 g $5L^{-1}$: Treatment 20 = End July, Early September, Mid October, SiTKO-SA 5 L ha^{-1} & Alga 600 3 g $5L^{-1}$: Treatment 21 = End July, Early September, Mid October, Bion 0.175 g L^{-1} & Regalia 2.5 L ha^{-1}: Treatment 22 = End July, Early September, Mid October, Regalia 2.5 L ha^{-1} & SiTKO-SA 5 L ha^{-1}. *** = P <0.001; ** = P <0.01; * = P <0.05

Supplementary Figure 4